Institut für Parasitologie
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# Epidemiology of the eye worm *Thelazia callipaeda* in cats from southern Switzerland, and therapeutic efficacy of milbemycin oxime/praziquantel oral formulation (Milbemax®) in naturally infested dogs and cats

**Inaugural-Dissertation**

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2014

Ai miei genitori e mia sorella

# Inhaltsverzeichnis

## Zusammenfassung

*Thelazia callipaeda* (ein Augenwurm) befällt u.a. Füchse, Hunde und Katzen und kann Konjunktivits, Keratitis oder Ulcera verursachen. Die Prävalenz von *T. callipaeda* bei 2171 Katzen, welche aus unterschiedlichen Gründen in einer Tierarztpraxis im Mendrisiotto (Südtessin) untersucht wurden, belief sich auf 0.8% (95% Konfidenzintervall: 0.5-1.3%). Männliche Katzen und Katzen älter als ein Jahr waren signifikant häufiger befallen. Die Mehrheit der Fälle wurde zwischen Juni und Dezember diagnostiziert. Im Durchschnitt beherbergten die Katzen 2.8 (1-10) Thelazien. Von den 17 positiven Katzen zeigten 3 milde Epiphora, 7 Konjunktivitis, und eine Katze Konjunktivitis und Ulcera, während 6 asymptomatisch waren. Die Studie bestätigt das endemische Vorkommen von *T. callipaeda* im Tessin und zeigt, dass der Befall bei Katzen häufig nicht identifiziert wird. Des Weiteren wurde die therapeutische Wirksamkeit von Milbemax® (Milbemycinoxim/Praziquantel) bei natürlich mit *T. callipaeda* infizierten Hunden und Katzen evaluiert. Hierfür wurden zwischen 2009 und 2011 56 Hunde und 31 Katzen aus dem Tessin (Südschweiz) und der Basilicata (Italien) am Tag 0 sowie, wenn noch positiv, 7 Tage später entweder mit Milbemax® oder mit Placebo behandelt. Die gute Wirksamkeit zeigte sich anhand einer im Vergleich zur Placebogruppe reduzierten Wurmbürde an den Tagen 7 bzw. 14 von 86.1% und 96.8% bei den Hunden und von 62.2% und 80.0 % bei den Katzen.

## Summary

*Thelazia callipaeda* (an eye worm) infests among others foxes, dogs, and cats, and may cause conjunctivitis, keratitis or ulcers. The prevalence of *T. callipaeda* in 2171 cats, which were examined for different reasons in a veterinary practice located in the Mendrisiotto region (southern Ticino), was 0.8% (95% Confidence Interval: 0.5-1.3%). Male cats and cats older than one year were significantly more often infested. The majority of cases were diagnosed between June and December. The cats harboured an average of 2.8 (1-10) eye worms. Among the 17 positive cats, 3 showed a mild epiphora, 7 conjunctivitis and one single cat conjunctivitis and ulcers, while 6 cats were asymptomatic. This study confirms the endemic presence of *T. callipaeda* in Ticino and shows that the infestation is often not identified in cats. Furthermore, the therapeutic efficacy of Milbemax® (milbemycin oxime/praziquantel) was tested in naturally infested dogs and cats. Between 2009 and 2011, 56 dogs and 31 cats from Ticino (southern Switzerland) and Basilicata (Italy) were treated at day 0, and, if still positive, again at day 7, either with Milbemax® or with placebo tablets. A high therapeutic efficacy was demonstrated by a worm count reductions, as compared to the placebo group, at day 7 and 14 of 86.1% and 96.8%, respectively, in dogs, and of 62.2% and 80.0% in cats.

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# Epidemiology of the eye worm *Thelazia callipaeda* in cats from southern Switzerland

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ABSTRACT

*Thelazia callipaeda* is a spiruroid nematode of dogs, cats and wild carnivores transmitted by zoophilic drosophilid *Phortica* flies and found in an increasing number of European countries. In cats the disease is diagnosed sporadically. This study presents an epidemiological investigation of feline thelaziosis, performed in southern Ticino, Switzerland, an endemic area for *T. callipaeda*. Between January 2009 and July 2011 2171 cats, having outdoor access and presenting for various reasons, were examined by in-depth eye examinations, and clinical and anamnestic data were collected. The overall prevalence of *T. callipaeda* in the study area was 0.8% (17/2171 cats, 95% confidence interval: 0.5–1.3%). Among cats showing ocular illness, the prevalence was 9.2% (11/120, CI: 4.7–15.8%). Cats with eye worms had no international travel history and were significantly more often diagnosed between June and December than during other months. With one exception, one single eye per cat was infested, each harboring between 1 and 10 eye worms (arithmetic mean: 2.8 per cat). One cat presented with conjunctivitis and ulcers, seven with conjunctivitis only and 3 with a mildly increased lacrimation, while 6 cats were asymptomatic. Significantly more male than female cats had eye worms and cats older than one year were overrepresented. No pure-bred cats were infested. This study confirms the establishment of this potentially zoonotic parasite in cats from the study area. Due to the clinical relevance and pain caused by the infestations, increased disease awareness and in depth eye examination for the detection of *T. callipaeda* in cats are recommended, even in absence of obvious clinical signs, in order to initiate appropriate anthelmintic treatment.



## 1. Introduction

*Thelazia callipaeda* (Spirurida, Thelaziidae) is a whitish nematode of 0.5–1.8 cm length infesting the conjunctival pouches and the lachrymal ducts of dogs, cats, rabbits, wild carnivores and humans (Anderson, 2000). Adult and pre-adult stages cause mechanical damage to the conjunctival and corneal epithelium (Otranto and Traversa, 2005), provoking lacrimation, conjunctivitis or even keratitis, uveitis and corneal ulcers (Bussiéras et al., 1996; Malacrida et al., 2008). Lacrimal secretions are of significance for the transmission of first stage larvae (L1) to the intermediate and

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vector host, the *Phortica* flies (Diptera, Drosophilidae). Attracted by ocular discharge, the flies ingest L1 while feeding on conjunctival secretions of an infested animal and, after approximately 3 weeks, they also transmit the infective third stage larvae (L3) to a definitive host (Otranto et al., 2004). Without treatment, adult worms can persist up to one year behind the third eyelid.

The past and present occurrence of this parasite in humans in far Eastern countries spread from India (Singh and Singh, 1993) to Japan (Koyama et al., 2000; Shen et al., 2006), led to the common name of "oriental eye worm" for *T. callipaeda*. Its presence is meanwhile increasingly reported also in carnivores in Europe, starting from 1989 (Rossi and Bertaglia, 1989). High prevalence is reported in dogs from Basilicata, in southern Italy (Otranto et al., 2003, 2009), where the density of *Phortica* flies is high and their seasonal activity is consistent between May and October (Otranto et al., 2006). However, the presence of adequate intermediate vectors has also been detected in Switzerland in the northern part of the Alps, with a typical continental climate (Roggero et al., 2010). At present, *T. callipaeda* is focally endemic in several European countries, infesting domestic and wild carnivores in Italy (Otranto et al., 2009) and southern Switzerland (Malacrida et al., 2008), domestic animals in France (Dorchies et al., 2007; Ruytoor et al., 2010), and, recently, on the Iberian peninsula (Miro et al., 2011; Vieira et al., 2012). A single infested dog without travel history was diagnosed in Germany (Magnis et al., 2010).

In previous studies conducted between 2005 and 2007 in the southern part of Switzerland, in the Canton of Ticino, which borders northern Italy, a prevalence of 6.2% and 11.1% has been detected in dogs and foxes, respectively (Malacrida et al., 2008). Contemporaneously, only five cats were diagnosed with *T. callipaeda* in the same area. In a field efficacy study performed in Italy and Switzerland, a total of 31 cats with *T. callipaeda* were identified (Motta et al., 2012). Previous studies reported sporadic cases in five cats in Italy, three in France and two in Portugal (Di Sacco et al., 1995; Dorchies et al., 2007; Otranto et al., 2003; Rodrigues et al., 2012; Ruytoor et al., 2010; Soares et al., 2013).

The aim of this study was to investigate epidemiological data concerning feline thelaziosis from a region known to be endemic for *T. callipaeda* in dogs and foxes.

## 2. Materials and methods

### 2.1. Animals and study area

Between January 2009 and July 2011 a total of 2171 cats were presented to a veterinary practice, located in southern Ticino (Switzerland), for different health concerns. The criteria for the cats to be enrolled in this study were to be client-owned, at least 6 weeks old and weighing more than 0.5 kg body weight (BW). All cats were living around the Mendrisiotto region (101 km$^2$, latitude 45°52 N 8°59 E, altitude ranging from 277 to 571 m above sea level), an area bordering northern Italy. The climate is characterized by a mean monthly relative humidity (RH) varying between 60% and 75% and varying monthly precipitations between 52 and 196 mm (see climate diagrams of Lugano and Stabio, www.meteoschweiz.admin.ch) and maximum monthly temperatures >20° C between May and September. Around 60.6% of the Mendrisiotto territory is wooded, 19.2% is agricultural, 18.8% is urban and 1.4% is unproductive surface (Poretti Suckow et al., 2012).

### 2.2. Procedures

All cats were subjected to an in-depth ocular examination in order to detect *T. callipaeda* infestations. Cats subjected to surgery were anesthetised adopting a combination of xylazin (2–4 mg/kg BW, Xylazin®, Streuli) and ketamine (S-Ketamin®, 3 mg/kg BW, Dr. E. Graeub) administered intramuscularly, allowing the third eyelid to be raised by means of ocular tweezers for visualization of the nematodes. The conjunctival pouches of cats not subjected to surgery were inspected after the application of two drops per eye of a local anesthetic (oxybuprocaine, Novesin® 0.4%, Omnivision).

The number of (pre-)adult worms was counted in each eye: if there were less than 10 worms, the exact number was counted and the infestation intensity was recorded as mild (1–5 worms), moderate (6–10 worms) and severe (>10 worms). Eye lesions such as conjunctivitis, lacrimation, ulcers, keratitis and ocular discharge were also recorded and classified as mild, moderate and severe. Some of the owners of cats harboring *T. callipaeda* were extensively informed about the parasite in the context of a placebo controlled, blinded field study (Motta et al., 2012). These cats were followed for a period of 14 days through weekly examinations.

### 2.3. Statistical analysis

Data about gender, age and breed were examined and correlated to the infestation with *T. callipaeda*. For statistical analysis cats were divided by sex, age (cats younger than one year versus cats older than one year) and 2 breed groups (European short hair and crossbreed cats versus purebred). The number and percentage of animals with ocular illness in each gender or age group were summarized in contingency tables and Fisher's Exact tests were applied for the statistical comparison between those groups, with the level of significance being $p < 0.05$. Tests were performed two-tailed. Fisher's Exact tests were performed with SAS® (SAS Institute Inc., Cary, NC, USA). Exact binomial 95% confidence intervals (CI) for means of binomial variables were calculated with unweighted data according to the method of Clopper and Pearson (1934). Graphics were performed using GraphPad Prism version 4.02 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

## 3. Results

### 3.1. Anamnestic data and reasons for consultation

All examined cats had access to both outdoors and indoors, were of either sex ($n = 1129$, 52% males, and $n = 1042$, 48% females) and predominantly European short hair (see Table 1). Reasons for presenting at vet practices

**Table 1**
Sex, age and health concerns of 2171 cats from the Mendrisiotto region (southern Switzerland) inspected for infestation with the eye worm *Thelazia callipaeda*.

| | All examined cats (*n* = 2171) | | | Cats with ocular illness (*n* = 120) | | | Cats with *T. callipaeda* infestation (*n* = 17) | | |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | % | 95% CI | *n* | % | 95% CI | *n* | % | 95% CI |
| Sex | | | | | | | | | |
| Male | 1129 | 52.0 | 49.9–54.1 | 60 | 50 | 40.7–59.3 | 13 | 76.5 | 50.1–93.2 |
| Female | 1042 | 48.0 | 45.9–50.1 | 60 | 50 | 40.7–59.3 | 4 | 23.5 | 6.8–49.9 |
| Age[a] | | | | | | | | | |
| <1 year | 302 | 13.9 | 12.5–15.4 | 20 | 16.7 | 40.5–24.6 | 1 | 5.9 | 0.1–28.7 |
| >1 year | 111 | 5.1 | 4.2–6.1 | 100 | 83.3 | 75.4–89.5 | 16 | 94.1 | 71.3–99.9 |
| Unknown | 1758 | 81.0 | 79.2–82.6 | n.a. | n.a. | | n.a. | n.a. | |
| Breed | | | | | | | | | |
| ESH[b]/crossbred | | | | 106 | 88.3 | 81.2–93.5 | 17 | 100 | 83.8–100.0 |
| Purebred | | | | 14[c] | 11.7 | 6.5–18.8 | 0 | 0 | 0–16.2 |
| Health concerns | | | | | | | | | |
| Ocular illness | 120 | 5.6 | 4.6–6.6 | n.a. | n.a. | n.a. | 8 | 47.1 | 23.0–72.2 |
| Vaccination | 1004 | 46.2 | 44.1–48.4 | n.a. | n.a. | n.a. | 0 | 0 | 0–16.2 |
| Spaying | 130 | 6.0 | 5.0–7.0 | n.a. | n.a. | n.a. | 0 | 0 | 0–16.2 |
| Neutering | 153 | 7.0 | 6.0–8.2 | n.a. | n.a. | n.a. | 0 | 0 | 0–16.2 |
| Other | 764 | 35.2 | 33.2–37.2 | n.a. | n.a. | n.a. | 9 | 52.9 | 27.8–77.0 |

[a] For more details on the age of cats with ocular illness and with *T. callipaeda* infestation see Fig. 2.
[b] ESH, European short hair.
[c] 7 Persian cats (2 neutered males, 3 neutered and 2 intact females); 3 Main Coon cats (all neutered males), 2 Ragdoll cats (1 neutered male and female respectively), one Sorian and a British Shorthair cat, both neutered males.

were vaccinations (1004), neutering (153), spaying (130) and specific consultations due to ocular problems (120). These included *T. callipaeda* affected cats with clinical signs (see Fig. 1). Other reasons included medical reasons (764) such as diarrhea, vomiting and apathy.

### 3.2. Cats with ocular illness

Cats with ocular illness were classified as follows: 80% ($n = 96$) showed conjunctivitis, 17.5% ($n = 21$) had ulcers, and 0.8% (one cat) had combined ulcer and conjunctivitis, while 1.7% ($n = 2$ cats) harbored *T. callipaeda* and showed very mild lacrimation detected at veterinary examination but unnoticed by their owners. There was no difference between the number of males and females affected, whilst in the age categories, differences were significant ($p = 0.002$), with animals younger than one year being less often presented for ocular illness. Fourteen cats with ocular illness were purebred (of which, 7 were Persians, see Table 1, note 3); none of them were harboring *T. callipaeda*. Among the animals with ocular illness, 9.2% (11/120, 95% CI: 4.7–15.8) were *T. callipaeda* positive.

### 3.3. Cats with *T. callipaeda*

Overall prevalence of feline thelaziosis was 0.8% (17/2171, 95% CI: 0.5–1.3%). Among the cats with *T. callipaeda* infestation, only 11 showed ocular clinical signs.

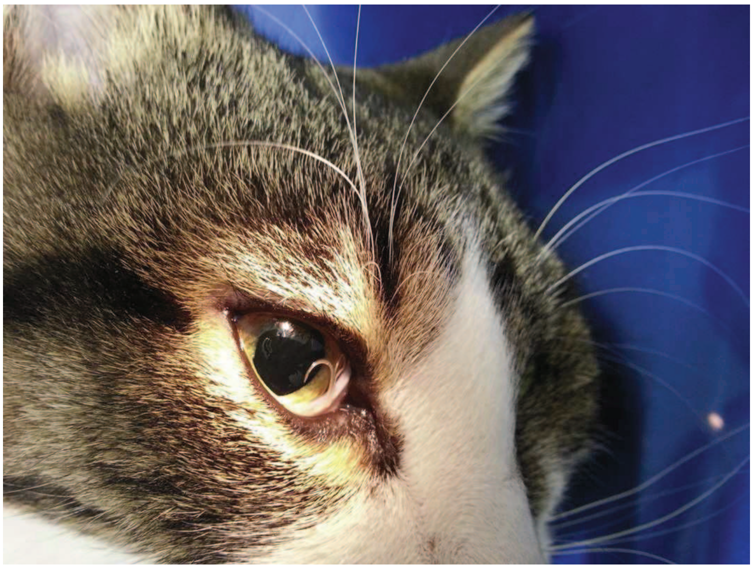

**Fig. 1.** *Thelazia callipaeda* in the eye of a cat from the Mendrisiotto region (Ticino, southern Switzerland) showing increased lacrimation.

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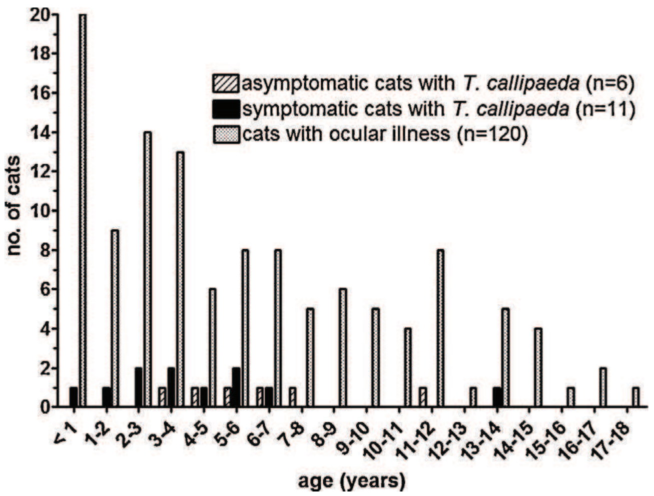


**Fig. 2.** Age of 120 cats examined for ocular concerns (dotted column) and of 17 cats harboring *Thelazia callipaeda* examined: 6 were asymptomatic (striped column) and 11 showed clinical signs (black column). Cats were visited between January 2009 and July 2011 in a veterinary practice in the Mendrisiotto region (southern Ticino, Switzerland).

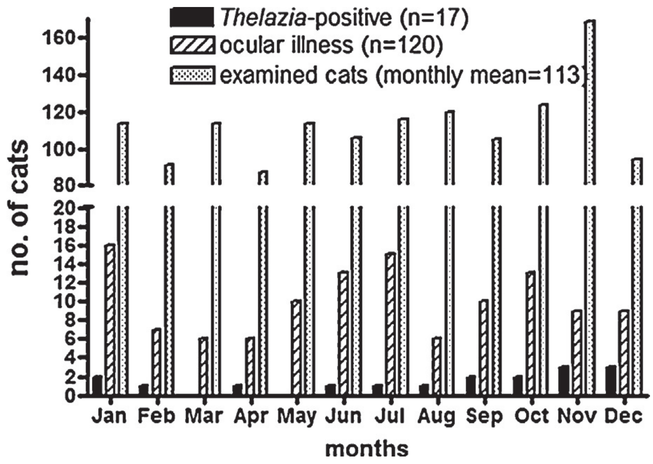


**Fig. 3.** Seasonal distribution of *Thelazia callipaeda* positive cats (black column) and of cats examined for other ocular problems (striped column) between January 2009 and July 2011 in a veterinary practice in the Mendrisiotto region (southern Ticino, Switzerland). These columns are itemized with the mean number of visited cats for each month during the same period in the same veterinary practice (dotted column).

Nine cats had conjunctivitis, of which one single cat also had ulcers in the same eye, the same cat as mentioned above; this neutered male, 8 year old, crossbred Norwegian cat harbored more than 10 *Thelazia* specimens. Two further already mentioned cats manifested mild lacrimation. A total of 9 of the *Thelazia* positive cats were presented by the animal owner for problems not connected with ophthalmic diseases. One cat was visited due to anorexia and fever, another due to cystitis, two cats due to abscesses, three for a health check-up and another one due to infection of the upper respiratory tract. This latter cat, together with a further cat from the region of Morbio Inferiore, was checked due to living with a *Thelazia* affected cat and dog respectively.

In contrast to the cats with ocular illness, where the ratio was balanced, significantly more male than female cats ($p = 0.029$) were infested with *T. callipaeda* (Table 1). One single animal (male cat) was intact, while the others were neutered. Furthermore, significantly more cats infested with *T. callipaeda* were older than one year (16/17 cats, 91.1%, CI: 71.3–99.9). More precisely, one single *Thelazia* positive cat was aged 8 months, two cats were older than 11 years, while the remaining 14 cats were aged between 2 and 7 years (Fig. 2). In contrast, the age of cats with ocular illness varied from cats younger than one year (20 out of 120, 16.7%) up to 18 years, with a peak in the former ones and a logarithmic decreasing trend ($R^2 = 0.8016$) with increasing age (Fig. 2). Significantly less purebred cats with ocular illness were examined, and no purebred cat was diagnosed with *T. callipaeda* infestation.

The number of *Thelazia* specimens per eye varied from 1 to more than 10 (arithmetic mean: 2.8 worms per eye). Most cats harbored 1–2 eye worms, only 2 cats had an infestation with 4 and 5 *Thelazia* specimens per eye, respectively, and in the single cat harboring more than 10 eye worms in the same eye the exact number was not determined (10 was used for calculations). Ten cats had ocular worms in the right eye, while 6 of them in the left eye. One single intact female cat had *Thelazia* specimens in both conjunctival pouches (2 worms in the right and 1 in the left eye).

None of the *Thelazia* affected cats had a history of travel to Italy or to other countries known to be endemic for *T. callipaeda*. Four cats were diagnosed during winter and spring (23.5%, 95% CI: 6.8–49.9) between January and May, while the other 13 cats were diagnosed between June and December (76.5%, 95% CI: 50.1–93.2), indicating a significant difference. This is in opposition to cats with ocular illness, which were diagnosed throughout the year, with highest numbers (16 cats) found in January (Fig. 3).

The geographical distribution of the cats with ocular illness is shown in Fig. 4. Cases are clustered in and around centers and residential areas, in correlation with the human, and therefore also the cat population. For a clearer presentation, 2 cases with ocular illness from northern Ticino are not shown. These 2 cats were both neutered males and one of them was *T. callipaeda* positive.

## 4. Discussion

Here we present epidemiological data about feline thelaziosis in a region known to be endemic for dogs and foxes. The occurrence of *T. callipaeda* in cats is expected in particular in regions with high prevalence in dogs or foxes, as confirmed while recruiting cats for field efficacy studies in Italy and Switzerland, where 20 and 11 cats respectively were diagnosed (Motta et al., 2012). The prevalence of 0.8% (95% CI: 0.5–1.3%) in cats as determined by this study appears to be low. A previous investigation performed in the same area evidenced a prevalence of 6.2% and 11.1% in dogs and foxes, respectively (Malacrida et al., 2008), while 5 cats were diagnosed between 2000 and 2007, presenting with conjunctivitis (2 cats) and keratitis (1 cat). The 17 positive cats of this study were diagnosed within a much shorter time. However, 9 of them were brought to the veterinarian for reasons not related to ocular problems and among these, 6 cats were asymptomatic. In these 9 cats, the infestation with *T. callipaeda* could have been missed out of

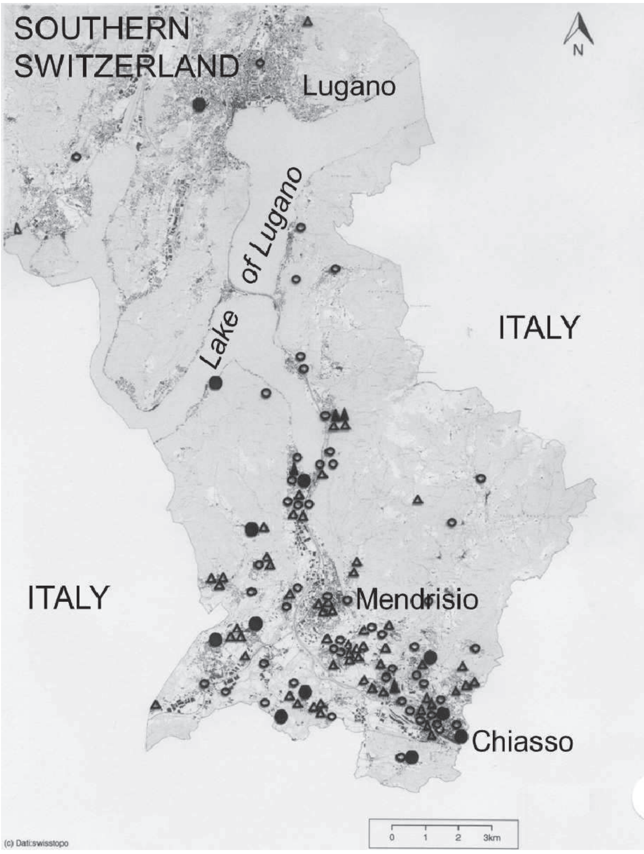


**Fig. 4.** Localization of 124 cats (60 females = triangles, 64 males = circles) visited between January 2009 and July 2011 in a veterinary practice in the Mendrisiotto region (southern Ticino, Switzerland). Symbols for 17 cats infested with the eye worm *Thelazia callipaeda* are filled in: 11 were showing ocular illness, 6 were asymptomatic. All other cats were presented for ocular illness not related to *T. callipaeda* infestation.

the context of this study. Cats generally showed poor or no clinical signs, confirming that such infestations may easily pass unobserved by cat owners, and also by veterinarians: in-depth eye inspection in cats is not a routine analysis for practitioners because cats need to be well sedated or anesthetized for appropriate examination, including lifting of the nictitating membrane, where the worms tend to hide. The limited clinical signs may be explained by the low number of eye worms in cats. In the previously mentioned study cats were harboring only 1–2 worms per eye (Malacrida et al., 2008). With a mean of 2.76 worms per eye, cats of this study seem to be more exposed to *Thelazia* infestations by trend. Furthermore, a single eye per cat was infested, with the exception of one cat having both eyes affected. In contrast, approximately one third of the dogs harbored eye worms in the left eye, one third in the right eye and one third in both eyes (Malacrida et al., 2008), suggesting that cat's eyes are at a lower risk of infestation than dog eyes.

If clinical signs are observed in cats, they seem to decrease with time of infestation, despite the persisting presence of the parasite; this reflects what was seen in the clinical follow-up study testing the efficacy of milbemycinoxime, where cats of the treated group did not clinically differ from the cats of the placebo group 7 days after treatment (Motta et al., 2012). It can therefore be assumed that prevalence in cats is generally underestimated, explaining why previous reports of *T. callipaeda* in cats are anecdotal and why data about thelaziosis in cats and correlated risk factors are scarce.

For risk factor analysis, the study population has to be considered: a selection of cats which had to be anesthetized for different reasons was undertaken. The reasons for the veterinary examination were highly variable; based on the large number and on anamnestic data, the selected 2171 cats are assumed to represent a random cat population coming to a veterinary practice. In this study population, the number of male and female cats was equivalent. Unfortunately, the age was only known for 413 cats, not allowing therefore an evaluation of the whole population. Few pure-bred cats were examined, making assertions concerning breed predisposal difficult. Intriguingly, none of the pure-bred cats was *T. callipaeda* positive, while 11.1% of the cats with ocular illness were purebred, suggesting that possibly pure-bred cats such as Persians are more often examined for ocular problems due to anatomic conditions of their ocular ducts, but not for *Thelazia*-infestations. In dogs, a breed-size predisposal was defined, with lower prevalence rates in small sized breeds, and in young dogs (Malacrida et al., 2008). It was argued that prevalence in cats may be lower compared to dogs because of their small body mass index, making them less attractive for *Phortica* flies (the vectors for *T. callipaeda*), and because cats usually have intensive cleaning habits eliminating eye discharges. In fact, *Phortica* flies feed on fruits as other drosophilid species, but they additionally are attracted by eyes of humans and other mammals (Otranto et al., 2006; Roggero et al., 2010), showing a zoophilic behavior. Moreover, flies were described to be attracted by decaying fruits or slime fluxes and also by lachrymal secretions (Bächli et al., 2004; Otranto et al., 2006). Due to these particular feeding preferences, we may argue that *Phortica* flies are especially attracted by eyes with ocular discharge, which is often increased in animals with ocular problems. However, it remains debatable if the vector is particularly attracted by ocular discharge or if ocular discharge is a consequence of *Thelazia* infestation.

Significantly more male than female cats were found to be *Thelazia* positive, while in dogs contradictory results concerning sex predisposal were observed (Malacrida et al., 2008; Otranto et al., 2003). Since all cats of this study had outdoor access, it could be assumed that male cats are at higher risk for *T. callipaeda* infestation, possibly because of a wider roaming area and/or longer outdoor stay for hunting and territorial behavior (Finkler et al., 2011; Loyd et al., 2013), leading them to higher infestation risk.

Young cats may be at lower risk because of restricted contact with *Phortica* flies in their immediate surroundings, and/or because of the approximate 2 month duration after vector contact required for development of adult worms. The increasing number of cases with increasing age (in particular up to 6 years), may be linked to increased opportunities to vector contacts and to a potentially cumulative presence of the parasite in cats' eyes over time. Additionally, deworming frequency may decrease with increasing age of cats: veterinary actions such as vaccinations and desexing, which are often accompanied by administration of anthelmintic treatments, are frequent in the first year of life, and anthelmintic treatments considered less necessary later on by the animal owners. Thus, established deworming protocols (www.esccap.ch) are not necessarily followed and according to this, a high number of adult cats may remain untreated. Furthermore, not all anthelmintics are efficacious against *T. callipaeda*: while intestinal helminths are eliminated applying compounds of the group of the benzimidazoles, quinolines, benzoles, depsipeptides, pyrimidines or macrocylic lactones, only representatives of the latter ones were positively evaluated for efficacy against *T. callipaeda* (Ferroglio et al., 2008; Motta et al., 2012).

In respect of the relatively short time from infestation until adult parasites can be found, and that infestation can remain unnoticed for some time, it is not surprising that positive cats were diagnosed almost throughout the year. However, significantly more cases were diagnosed from June to December than during winter and spring, which is in contrast with the cases of ocular illness, which were more evenly distributed over the year. This difference indicates that the extent of the vector transmission season influences the seasonal detection of *T. callipaeda* positive cats. In southern Switzerland *Phortica* specimens were captured between April and October with the highest abundance of *Phortica* flies detected within an orchard in central Ticino (Roggero et al., 2010). In the studied Mendrisiotto region almost 20% is agricultural, which includes a considerable part of vineyard. This sustains the hypothesis that cases of *T. callipaeda* emerge in particular in areas attractive for the development of *Phortica* flies, as it has been shown in regions with fruit abundance, such as strawberry fields (Ruytoor et al., 2010).

Considering the potential development of uveitis or keratitis and correlated painful conditions due to the high innervation with sensory nerve fibers of the superficial cornea (Chan-Ling, 1989; Thomson et al., 2013) where *T. callipaeda* reside, and considering the potential role of cats in the transmission to the vector, it is important to detect such infestations for appropriate elimination. Currently, the therapy of *T. callipaeda* affected animals consists of the mechanical removal of the parasites and topical (Bianciardi and Otranto, 2005) or systemic (Motta et al., 2012) administration of macrocyclic lactones. Monthly anthelmintic treatments, which are advised as a control strategy for dirofilarioses and other helminth infections (see www.esccap.org), have already been suggested for animals living in areas endemic for *T. callipaeda* (Motta et al., 2012). Disease awareness among animal owners and veterinarians has likely increased ever since the first cases of thelaziosis were detected. Nevertheless, more frequent controls in all cats with outdoor access, in particular in male cats and in cats older than one year, are indicated for the detection of this underestimated disease, also in absence of clinical signs.

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## References

Anderson, R.C., 2000. Nematode Parasites of Vertebrates. Their Development and Transmission, 2nd ed. CABI publishing, Guilford, pp. 404–407.

Bächli, G., Vilela, C.R., Andersson Escher, S.A.S., 2004. The Drosophilidae (Diptera) of Fennoscandia and Denmark, Vol. 39. Brill, Leiden, New York, pp. 362.

Bianciardi, P., Otranto, D., 2005. Treatment of dog thelaziosis caused by *Thelazia callipaeda* (Spirurida Thelaziidae) using a topical formulation of imidacloprid 10% and moxidectin 2.5%. Vet. Parasitol. 129, 89–93.

Bussiéras, J., Chermette, R., Seillier, A.-M., 1996. Quelques parasitoses canines exceptionelles en France. II – Un cas de conjonctivite parasitaire du chien, due à Thelazia sp. Prat. Méd. Chir. Anim. Comp. 31, 83–85.

Chan-Ling, T., 1989. Sensitivity and neural organization of the cat cornea. Invest. Ophthalmol. Vis. Sci. 30, 1075–1082.

Clopper, C.J., Pearson, E.S., 1934. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 26, 404–413.

Di Sacco, B., Ciioccca, A., Sirtori, G., 1995. *Thelazia callipaeda* (Ralliet and Henry, 1910) nel sacco congiuntivale di un gatto di Milano. Veterinaria 9, 81–84.

Dorchies, P., Chaudieu, G., Siméon, L.A., Cazalot, G., Cantacessi, C., Otranto, D., 2007. First reports of autochthonous eyeworm infection by *Thelazia callipaeda* (Spirurida Thelaziidae) in dogs and cat from France. Vet. Parasitol. 149, 294–297.

Ferroglio, E., Rossi, L., Tomio, E., Schenker, R., Bianciardi, P., 2008. Therapeutic and prophylactic efficacy of milbemycin oxime (Interceptor) against *Thelazia callipaeda* in naturally exposed dogs. Vet. Parasitol. 154, 351–353.

Finkler, H., Gunther, I., Terkel, J., 2011. Behavioral differences between urban feeding groups of neutered and sexually intact free-roaming cats following a trap-neuter-return procedure. J. Am. Vet. Med. Assoc. 238, 1141–1149.

Koyama, Y., Ohira, A., Kono, T., Yoneyama, T., Shiwaku, K., 2000. Five cases of thelaziasis. Br. J. Ophthalmol. 84, 441.

Loyd, K.A., Hernandez, S.M., Abernathy, K.J., Shock, B.C., Marshall, G.J., 2013. Risk behaviours exhibited by free-roaming cats in a suburban US town. Vet. Rec. 173, 295.

Magnis, J., Naucke, T.J., Mathis, A., Deplazes, P., Schnyder, M., 2010. Local transmission of the eye worm *Thelazia callipaeda* in southern Germany. Parasitol. Res. 106, 715–717.

Malacrida, F., Hegglin, D., Bacciarini, L., Otranto, D., Nageli, F., Nageli, C., Bernasconi, C., Scheu, U., Balli, A., Marenco, M., Togni, L., Deplazes, P., Schnyder, M., 2008. Emergence of canine ocular Thelaziosis caused by *Thelazia callipaeda* in southern Switzerland. Vet. Parasitol. 157, 321–327.

Miro, G., Montoya, A., Hernandez, L., Dado, D., Vazquez, M.V., Benito, M., Villagrasa, M., Brianti, E., Otranto, D., 2011. *Thelazia callipaeda*: infection in dogs: a new parasite for Spain. Parasit. Vectors 4, 148.

Motta, B., Schnyder, M., Basano, F.S., Nageli, F., Nageli, C., Schiessl, B., Mallia, E., Lia, R.P., Dantas-Torres, F., Otranto, D., 2012. Therapeutic efficacy of milbemycin oxime/praziquantel oral formulation (Milbemax®) against *Thelazia callipaeda* in naturally infested dogs and cats. Parasit. Vectors 5, 85.

Otranto, D., Cantacessi, C., Testini, G., Lia, R.P., 2006. Phortica variegata as an intermediate host of *Thelazia callipaeda* under natural conditions: evidence for pathogen transmission by a male arthropod vector. Int. J. Parasitol. 36, 1167–1173.

Otranto, D., Dantas-Torres, F., Mallia, E., Digeronimo, P.M., Brianti, E., Testini, G., Traversa, D., Lia, R.P., 2009. *Thelazia callipaeda* (Spirurida Thelaziidae) in wild animals: report of new host species and ecological implications. Vet. Parasitol. 166, 262–267.

Otranto, D., Ferroglio, E., Lia, R.P., Traversa, D., Rossi, L., 2003. Current status and epidemiological observation of *Thelazia callipaeda* (Spirurida, Thelaziidae) in dogs, cats and foxes in Italy: a "coincidence" or a parasitic disease of the Old Continent? Vet. Parasitol. 116, 315–325.

Otranto, D., Lia, R.P., Buono, V., Traversa, D., Giangaspero, A., 2004. Biology of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms in naturally infected definitive hosts. Parasitology 129, 627–633.

Otranto, D., Traversa, D., 2005. *Thelazia* eyeworm: an original endo- and ecto-parasitic nematode. Trends Parasitol. 21, 1–4.

Poretti Suckow, D., Bottinelli, L., Paganetti, S., Stanga, M., 2012. Annuario statistico ticinese 2012, Repubblica e Cantone Ticino, D.d.f.e.d.e.,. Ufficio di statistica, ed. (Giubiasco, Società d'arti grafiche Veladini Lugano), 62.

Rodrigues, F.T., Cardoso, L., Coutinho, T., Otranto, D., Diz-Lopes, D., 2012. Ocular thelaziosis due to *Thelazia callipaeda* in a cat from northeastern Portugal. J. Feline Med. Surg. 14, 952–954.

Roggero, C., Schaffner, F., Bachli, G., Mathis, A., Schnyder, M., 2010. Survey of *Phortica* drosophilid flies within and outside of a recently identified transmission area of the eye worm *Thelazia callipaeda* in Switzerland. Vet. Parasitol. 171, 58–67.

Rossi, L., Bertaglia, P.P., 1989. Presence of *Thelazia callipaeda* Railliet & Henry 1910, in Piedmont, Italy. Parassitologia 31, 167–172.

Ruytoor, P., Dean, E., Pennant, O., Dorchies, P., Chermette, R., Otranto, D., Guillot, J., 2010. Ocular thelaziosis in dogs, France. Emerg. Infect. Dis. 16, 1943–1945.

Shen, J.L., Gasser, R.B., Chu, D., Wang, Z.X., Yuan, X., Cantacessi, C., Otranto, D., 2006. Human thelaziosis – a neglected parasitic disease of the eye. J. Parasitol. 92, 872–875.

Singh, T.S., Singh, K.N., 1993. Thelaziasis: report of two cases. Br. J. Ophtalmol. 77, 528–529.

Soares, C., Sousa, S.R., Anastacio, S., Matias, M.G., Marques, I., Mascarenhas, S., Vieira, M.J., de Carvalho, L.M., Otranto, D., 2013. Feline thelaziosis caused by *Thelazia callipaeda* in Portugal. Vet. Parasitol. 196, 528–531.

Thomson, S.M., Oliver, J.A., Gould, D.J., Mendl, M., Leece, E.A., 2013. Preliminary investigations into the analgesic effects of topical ocular 1% morphine solution in dogs and cats. Vet. Anaesth. Analg. 40, 632–640.

Vieira, L., Rodrigues, F.T., Costa, A., Diz-Lopes, D., Machado, J., Coutinho, T., Tuna, J., Latrofa, M.S., Cardoso, L., Otranto, D., 2012. First report of canine ocular thelaziosis by *Thelazia callipaeda* in Portugal. Parasit. Vectors 5, 124.

Motta et al. Parasites & Vectors 2012, 5:85
http://www.parasitesandvectors.com/content/5/1/85

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RESEARCH Open Access

# Therapeutic efficacy of milbemycin oxime/praziquantel oral formulation (Milbemax®) against *Thelazia callipaeda* in naturally infested dogs and cats

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## Abstract

**Background:** Over the last few decades, canine and feline thelaziosis caused by *Thelazia callipaeda* eye worms has gained the attention of the veterinary community due to the spread of this ocular infestation in geographical areas previously regarded as non endemic. The therapeutic efficacy of milbemycin oxime/praziquantel tablets (Milbemax®) against *T. callipaeda* was tested in naturally infested dogs and cats.

**Methods:** From January 2009 to July 2011 a placebo controlled and randomized field study was conducted in *T. callipaeda* endemic areas of Switzerland (CH) and Italy (ITA) involving client-owned animals. Dogs (n = 56) and cats (n = 31) were physically examined at enrolment Day 0 (D0) and twice afterwards (D7 and D14). Infested animals were orally treated with Milbemax® or with placebo tablets on D0 and, if an animal was found still infested with *T. callipaeda*, also on D7. On D14 nematodes were flushed from the conjunctiva, identified and counted.

**Results:** Out of 56 dogs, 43 were included in the statistical analysis, whereas 13 were excluded because the products under investigation were not administered with food, as required by the label. On D7 and D14, 72.7% and 90.9% of treated dogs were eye worm free, whereas in the placebo group 95.2% and 76.2% still harbored nematodes, resulting in a mean percentage worm count reduction for the Milbemax® group of 86.1% and 96.8%, respectively. Both results were significantly higher ($p = 0.0001$) than the placebo group. Out of the 31 cats included in the study at D7 and D14, 53.3% and 73.3% treated with Milbemax® were free of *T. callipaeda*, while 81.3% and 73.3 in the placebo group were still harbouring eye worms, resulting in a mean percentage worm count reduction for the treated group of 62.2% and 80.0%, respectively. Both results were significantly higher ($p = 0.0106$ and $p = 0.0043$) than the placebo group.

**Conclusions:** The commercial formulation of milbemycin oxime at the minimal dose of 0.5 mg/kg and 2 mg/k in dogs and cats, respectively, showed a high therapeutic efficacy in curing *T. callipaeda* infestations. The advantages of an oral application are additionally increased by the large spectrum of activity of praziquantel and milbemycin oxime against Cestodes and Nematodes infesting dogs and cats.

**Keywords:** *Thelazia callipaeda*, Milbemycin oxime, Dogs, Cats, Treatment

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## Background

*Thelazia callipaeda* (Spirurida, Thelaziidae) is a nematode infesting the eyes of dogs, cats, rabbits, wild carnivores and humans [1]. This parasite has been commonly known as the "oriental eye worm" because of its occurrence, mostly in human beings, in far Eastern countries such as Thailand [2], China [3] and Japan [4]. Nowadays, it is evident that *T. callipaeda* is also endemic throughout Europe infesting domestic and wild carnivores in Italy [5,6] and Switzerland [7], and domestic animals in France [8,9], Germany [10,11], and Spain [12]. Importantly, human cases of thelaziosis in Europe have been recorded in Italy and France [13].

Since the incrimination of *Phortica variegata* (Diptera, Drosophilidae) as a vector of *T. callipaeda* in Europe under laboratory [14] and natural conditions [15], the knowledge on this nematode and its vector has been greatly enhanced. The adult whitish nematodes (about 0.5–2 cm) and first stage larvae (L1) localize under the third eyelid provoking lacrimation, conjunctivitis or even keratitis and corneal ulcer [16]. L1s are released by the adult worms into the conjunctival secretions of infested animals and they are ingested by *P. variegata* flies while feeding on animal eyes, developing into the infective third stage larvae (L3) within about 3 weeks [17].

The parasitic stages of *T. callipaeda* (i.e., adults and larvae) may be removed mechanically by rinsing the conjunctival sac with sterilized saline fluids or by collecting the adults with fine forceps or cotton swab; however, worm removal may be incomplete. Antiparasitic drugs, such as macrocyclic lactones (e.g., moxidectin) have been proven efficacious in treating thelaziosis by ocular instillation [18,19]. For compliance reasons it may be recommended to use systemic macrocyclic lactones licensed for dogs and cats, such as the spot-on formulation containing moxidectin (Advocate®, Bayer HealthCare AG) [20], or oral formulations containing milbemycin oxime (Interceptor®, Milbemax®, Program Plus®, Sentinel®, Novartis Animal Health) [21]. Interceptor® showed a good therapeutic and prophylactic efficacy in treating thelaziosis in naturally infested dogs [21].

Due to the increasing attention of pet owners and practitioners on canine and feline thelaziosis and to the spread of this ocular infestation in geographical areas previously regarded as non endemic, new therapeutic options are solicited. Thus, the aim of this work was to evaluate the therapeutic efficacy of a commercial oral formulation of milbemycin oxime/praziquantel (Milbemax® - Novartis Animal Health) in dogs and cats naturally infested with *T. callipaeda*.

## Methods

The efficacy of Milbemax® (Novartis Animal Health) was evaluated in a placebo controlled, multicentric, blinded and randomized field study conducted in Switzerland (CH) in the Mendrisiotto region (Southern Ticino, 101 km$^2$, latitude 45°52 N and latitude 8°59 E, altitude ranging from 277–571 m above sea level), and in Italy (ITA) in the Basilicata region (Southern Italy, 9'992 km$^2$, latitude: 30° and 41°N; longitude 15° and 16° E, altitude ranging from 548–1367 m asl).

Dogs (Figure 1) and cats (Figure 2) naturally infested with *T. callipaeda* were enrolled from January 2009 to June 2011 (CH) and from October 2010 to July 2011 (ITA). The study was conducted under Good Clinical Practice, according to EMEA VICH GL9, GL7 and GL19. The trial was performed after obtaining written animal owner consent, animal trial permissions of the Ticino cantonal (Switzerland) veterinary office (permission numbers 04/2009 and 05/2009) and of the Italian authorities (permission numbers MoH Italy n. DGSA 0018416-P-14/10/2010).

### Animals

All dogs and cats were client-owned, living in *T. callipaeda* endemic areas, of both sexes, various breeds, at least 6 weeks old and weighing 0.5 kg or more at time of inclusion. Animals were required to be infested with a minimum of one worm in one conjunctival pouch and living outdoors or regularly going outdoors. Testing for infestation of *Dirofilaria immitis* was done in Southern Ticino, being a heartworm endemic area, prior to inclusion in accordance with Milbemax® label content. Only *D. immitis* negative dogs were included in the study.

### Procedures

Dogs and cats were physically examined by the veterinarian at enrolment (D0) and then at two follow-up visits, (D7 and D14). A general physical examination was

**Figure 1 Conjunctivits in a dog with *Thelazia callipaeda*.** Adult specimens of *Thelazia callipaeda* provoking conjunctivitis and mucopurulent discharge in the eye of a dog from Italy (Basilicata).

**Figure 2** ***Thelazia callipaeda*** **in the eye of a cat.** Eye of a cat from Basilicata infested with several adult specimens of *Thelazia callipaeda*.

carried out at each visit to determine the health status of the animals. This examination also included body weight (b.w.) determination on D0 and D14. A blood sample was collected on day 0, before the treatment, for baseline haematology and clinical chemistry. In Southern Ticino the presence of *D. immitis* antigen (Dirocheck$^{(R)}$, Synbiotics) and microfilariae (Knott's test) was assessed in dogs. At each visit, both eyes were examined for the presence of eye worms by clinical inspection of the conjunctival pouch, including a thorough examination underneath the third eyelid using a cotton swab. If necessary, 2 drops per eye of a local anaesthetic (oxybuprocaine hydrochloride solution, Novesin® 0.4%, Omnivision) were applied into the conjunctival pouches. Clinical signs indicative of eye worm infestation (e.g., lacrimation, conjunctivitis, ocular discharge, keratitis, ulcers) were recorded and classified as absent mild, moderate or severe. A fluorescein test to diagnose ulcers was only performed if the animal was suspected to have ulcers. Worms were counted in each eye separately and the infestation intensity categorized into very mild (1 worm), mild (2–5 worms), moderate (6–10 worms) and severe (>10 worms).

On D0 dogs and cats were randomly allocated to treatment or control group by a random treatment allocation plan and orally treated according to body weight, following the label instructions of the commercial formulations of Milbemax® in both countries. In order to keep the blinding on the treatment details, even though both products (i.e., the milbemycinoxime/praziquantel and the placebo tablets) were comparable in appearance, a technician, different from the veterinarian that performed clinical evaluations, was responsible for the administration of the product and the storage of all test product related documentation.

On D7, if an animal was still found to be infested with *T. callipaeda*, a re-treatment with the same product at the same dose was administered. On D14, at the final visit, the conjunctival pouches were flushed with 5 ml of saline solution (0.9% NaCl) to collect larval stages of the parasite that were identified and counted, following centrifugation (5 min at 2000 g) and microscopic examination (40×).

During the study period animals were observed daily by their owners for health abnormalities and physically examined by the veterinarian in case of adverse events. If eye worms were still detected after D14, parasites were mechanically removed or animals were treated with an injectable solution of moxidectin 1 per cent (Cydectin®, Fort Dodge Animal Health) administered by ocular administration as previously described [18].

### Statistical analysis

Data were statistically examined using SAS® Version 9.2 (SAS Institute, Cary, NC, USA). Summary statistics including arithmetic and geometric mean, minimum, maximum and median were provided for all counts, percentages or continuous parameters of interest. Primary efficacy objective was to compare the Milbemax® group with the placebo group with respect to clinical cure (therapeutic efficacy), i.e., complete elimination of adult eye worms, seven and fourteen days after treatment. Secondary efficacy objective was to compare the treatment groups with respect to worm count reduction and reduction of severity and/or presence of clinical signs caused by eye worm infestation. Worm count reduction was calculated for each animal as follows: % reduction$[t] = 100 \times (WC[t_o] - WC[t])/WC[t_o]$, where $WC[t_o]$ = baseline worm count before treatment and $WC[t]$ = worm count at time t after treatment. Fisher's exact test was applied for the statistical comparison of clinical cure rates and infestation frequencies between treatment groups at different study days. Worm count reduction percentages were analyzed by analysis of variance methods if the assumption of normal distribution was satisfied on original scale or after transformation. Otherwise, Mann–Whitney *U* test was performed to compare the treatment groups. Adverse events observations were summarized and Fisher's exact test was applied for the statistical comparison between groups. The level of significance was set at $p = 0.05$. Tests were performed two-sided.

## Results

### Dogs

Out of 56 dogs, which fulfilled the inclusion criteria, 43 (19 CH, 23 ITA) were included in the statistical analysis. Thirteen dogs were excluded because the investigational products (Milbemax® or placebo) were administered without any food, thus not as per the recommendations on the package leaflet. The included dogs were between

**Table 1 Worm count reduction in dogs infested with *Thelazia callipaeda* after oral treatment with milbemycin oxime/praziquantel**

| | Mean number of worms per dog (n, range) | Animals without worms (n, %) | | Worm count reduction (%) | |
|---|---|---|---|---|---|
| | Study day 0 | Study day 7 | Study day 14 | Study day 7 | Study day 14 |
| Milbemax® group (n = 22) | 6.14 (1–22) | 16 (72.7%)* | 20 (90.9%)* | 86.1%* | 96.8%* |
| Placebo group (n = 21) | 6.0 (1–22) | 1 (4.8%) | 5 (23.8%) | 10.8% | 27.5% |

* significant difference between milbemycin oxime/praziquantel-group and placebo-group.

2 months and 13 years old, 26 were males (19 intact, 7 neutered) and 17 were females (12 intact, 5 spayed) and they were of various breeds (n = 24), including crossbreds (n = 19). Data on the worm count reductions are reported in Table 1. On D0, dogs treated with Milbemax® (n = 22) and dogs of the placebo group (n = 21) harbored a mean (arithmetic) of 6.14 and of 6.0 worms, respectively. The percentage of animals harbouring worms after treatment was significantly different ($p = 0.0001$) between the groups, in favor of the Milbemax® treated group. The mean percentage worm count reduction was significantly higher for the Milbemax® group than for the placebo group ($p = 0.0001$) on D7 and D14. The mean number of worms harbored by treated dogs was 1.36 and 0.14 on D7 and D14, respectively, while worm burdens of dogs of the placebo group were 5.71 and 5.38, respectively, with these differences being significant ($p = 0.0001$) at D7 and D14, but not at D0 ($p = 0.4054$). At D14 *T. callipaeda* larval stages were detected only in 3 dogs of the placebo group. Frequencies and percentages of clinical parameters indicative of eye worm infestation were not different between the groups.

### Cats

A total of 31 cats (11 CH, 20 ITA) aging between 8 months and 14 years old, corresponded to the inclusion criteria and were included in the analysis. Of these, 19 were males (10 intact, 9 neutered) and 12 females (11 intact, one spayed). Data on the worm count reductions are reported in Table 2. At Day 0, treated (n = 15) and untreated (n = 16) cats harboured a mean (arithmetic) of 2.40 and of 2.38 worms, respectively. At D14, the number of animals free of *T. callipaeda* was higher in the treated group, with this difference being significant ($p = 0.0268$). The mean percentage worm count reduction for the Milbemax® group was significantly higher ($p = 0.0106$ and $p = 0.0043$) than the ones of the placebo group, on D7 and D14, respectively. The worm counts for the Milbemax® group were significantly lower than for the placebo group at D7 (0.93, $p = 0.230$) and 14 (0.80, $p = 0.0129$), while there was no significant difference on D0 ($p = 0.4530$). In 3 cats from the placebo group, larval stages could be found on D14. The presence of lacrimation on D0 was registered for 26.7% and 37.5% of the cats from the Milbemax® and the placebo group, respectively. This difference was not significant ($p = 0.7043$).

On D7 lacrimation was detected in 6.7% and 43.8% of the cats of the Milbemax® and of the placebo group, with this difference being significant ($p = 0.0373$), in contrast to data obtained on D0 ($p = 0.7043$).

## Discussion

The commercial formulation of milbemycin oxime (Milbemax®) at the minimal dose of 0.5 mg/kg and 2 mg/kg b.w. for dogs and cats, respectively, also containing praziquantel (5 mg/kg b.w.), showed a high therapeutic efficacy in curing *T. callipaeda* in naturally infested animals. In dogs the efficacy was 72.7% and 90.9% after a single or two treatments, at a weekly interval, both significantly differing from the placebo group. In cats, the therapeutic efficacy was 53.3% and 73.3% after a single or two treatments, at a weekly interval. It is known that praziquantel is not efficacious against nematodes. Since both actives of Milbemax®, milbemycin oxime and praziquantel, are well established on the market and known not to interfere with each other, it can be assumed that other formulations containing milbemycin oxime alone or in combination with other actives (e.g., Interceptor®, Sentinel®, Sentinel Spectrum® and Program Plus®; all Novartis Animal Health) may be efficacious against *T. callipaeda*. Furthermore,

**Table 2 Worm count reduction in cats infested with *Thelazia callipaeda* after oral treatment with milbemycin oxime/praziquantel**

| | Mean number of worms per cat (n, range) | Animals without worms (n, %) | | Worm count reduction (%) | |
|---|---|---|---|---|---|
| | Study day 0 | Study day 7 | Study day 14 | Study day 7 | Study day 14 |
| Milbemax® group (n = 15) | 2.40 (1–11) | 8 (53.3%) | 11 (73.3%)* | 62.2%* | 80.0%* |
| Placebo group (n = 16) | 2.38 (1–9) | 3 (18.8%) | 4/15 (26.7%) | 20.0% | 28.0% |

* significant difference between Milbemax®-group and placebo-group.

since *T. callipaeda* lives in the conjunctival pouches of the final host, an accurate dosing to ensure optimal blood concentrations of milbemycin oxime is needed to reach efficacious concentrations of the product in the conjunctives. This may explain the reasons for a higher efficacy of the product after a second treatment one week after the first, as also suggested by the results of a preliminary study on *T. callipaeda* naturally infested animals [21]. A lower efficacy observed on the 13 dogs (excluded from the statistical analysis) in which the treatment was administered without food, highlights the importance of a correct administration of the drug.

This study first evaluated the therapeutic efficacy of milbemycin oxime and praziquantel against *T. callipaeda* in cats. In addition, on the basis of a previous study in which the monthly administration of milbemyin oxime in dogs was highly effective (96.7%) for the prophylaxis of *T. callipaeda* [21], it may be argued that a similar prophylactic effect might occur in cats. This hypothesis deserves to be further tested under field conditions. Treatment of thelaziosis is an important issue in animals living in endemic areas, such as Basilicata in Italy (prevalence up to 60%; [6]), or Spain (prevalence of 39.9%; [12]) and Switzerland (prevalence up to 5%; [7]). An efficacious treatment against *T. callipaeda* is useful for pet owners considering the spread of the parasite in areas previously regarded as non endemic, such as France [9] and Spain [12].

The reasons for such an increase in cases of thelaziosis in dogs and cats throughout Europe are unknown, but it could be related to the spread of vector populations as well as to the occurrence of the infestation in wildlife species (e.g., foxes, wolves, beech martens and brown hares), which act as reservoirs for *T. callipaeda* [5]. Therefore, domestic animals which are traveling together with their owners from non-endemic to areas endemic for *T. callipaeda* should be treated since they are at risk of acquiring thelaziosis, as reported for some dogs in France or Germany [10,22]. Thus, monthly anthelmintic treatments, which are already recommended as a control strategy for dirofilarioses and other helminth infestations (e.g. see ESCCAP.org), should be considered for animals living in areas endemic for *T. callipaeda* in order to eliminate larval stages soon after their transmission from the drosophilid flies, thus interrupting the host-parasite transmission chain [17,23]. The high level of efficacy demonstrated in the current study suggests that further investigations should be carried out in order to test the effectiveness of the product when administered monthly during the risk season, in preventing *T. callipaeda* infestations in dogs and cats.

In addition, since Milbemax®-tablets for cats are flavor coated and chewable tablets are available for dogs, the oral formulations are very easy to apply, compared to the non-licensed local instillation of antiparasitic drugs [18,19] or to the mechanical removal of parasites from eyes. This especially applies when dealing with non-cooperative dogs and cats, where restraining them for manipulations around the eyes or even for spot-on applications is particularly difficult and bears the risk of trauma. Furthermore, wet coats or rainy days are reported to reduce the efficacy of topical administrations [20], a problem that is avoided by the drug administration *per os*.

## Conclusion

The commercial formulation of milbemycin oxime at the minimal dose of 0.5 mg/kg and 2 mg/kg milbemycin oxime for dogs and cats, respectively, showed a high therapeutic efficacy in curing *T. callipaeda* infestations. The advantages of an oral application of Milbemax® are additionally increased by the large spectrum of activity of praziquantel and milbemycin oxime against Cestodes and Nematodes infesting dogs and cats.

#### Competing interests

The authors declare that they have no competing interests.

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BM participated in the design of the study and in the field work, carried out the diagnostic assays and participated in the elaboration of the manuscript. MS and DO coordinated the study, participated in its design, in the field work and drafted the manuscript. FSB monitored the field studies, participated in the evaluation of the study results, helped to draft the manuscript and took the two pictures. FN, CN, RPL, FD-T and EM participated in the field studies sampling the animals. BS participated in the study design, coordinated the study participants and the statistical analysis and helped to draft the manuscript. All authors read and approved the final manuscript.

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#### References

1. Anderson RC: ***Nematode parasites of vertebrates***. UK: Their development and transmission. CABI publishing Guilford; 2000:404–407.
2. Bhaibulaya M, Prasertsilpa S, Vajrasthira S: ***Thelazia callipaeda* Railliet and Henry, 1910, in man and dog in Thailand.** *AmJTrop Med Hyg* 1970, **19:**476–479.
3. Shi YE, Han JJ, Yang WY, Wei DX: ***Thelazia callipaeda* (Nematoda: Spirurida): transmission by flies from dogs to children in Hubei, China.** *Trans Royal Soc Trop Med Hyg* 1988, **82:**627.

4. Koyama Y, Ohira A, Kono T, Yoneyama T, Shiwaku K: **Five cases of thelaziasis.** *British J Ophthalm* 2000, **84:**441.
5. Otranto D, Dantas-Torres F, Mallia E, Digeronimo PM, Brianti E, Testini G, Traversa D, Lia RP: ***Thelazia callipaeda*** **(Spirurida, Thelaziidae) in wild animals: Report of new host species and ecological implications.** *Vet Parasitol* 2009, **166:**262–267.
6. Otranto D, Ferroglio E, Lia RP, Traversa D, Rossi L: **Current status and epidemiological observation of** ***Thelazia callipaeda*** **(Spirurida, Thelaziidae) in dogs, cats and foxes in Italy: a "coincidence" or a parasitic disease of the Old Continent?.** *Vet Parasitol* 2003, **116:**315–325.
7. Malacrida F, Hegglin D, Bacciarini L, Otranto D, Nageli F, Nageli C, Bernasconi C, Scheu U, Balli A, Marenco M, *et al*: **Emergence of canine ocular thelaziosis caused by** ***Thelazia callipaeda*** **in southern Switzerland.** *Vet Parasitol* 2008, **157:**321–327.
8. Dorchies P, Chaudieu G, Siméon LA, Cazalot G, Cantacessi C, Otranto D: **First reports of autochthonous eyeworm infection by** ***Thelazia callipaeda*** **(Spirurida, Thelaziidae) in dogs and cat from France.** *Vet Parasitol* 2007, **149:**294–297.
9. Ruytoor P, Dean E, Pennant O, Dorchies P, Chermette R, Otranto D, Guillot J: **Ocular thelaziosis in dogs, France.** *Emerg Infect Dis* 2010, **16:**1943–1945.
10. Hermosilla C, Herrmann B, Bauer C: **First case of** ***Thelazia callipaeda*** **infection in a dog in Germany.** *Vet Rec* 2004, **154:**568–569.
11. Magnis J, Naucke TJ, Mathis A, Deplazes P, Schnyder M: **Local transmission of the eye worm** ***Thelazia callipaeda*** **in southern Germany.** *Parasitol Res* 2010, **106:**715–717.
12. Miro G, Montoya A, Hernandez L, Dado D, Vazquez MV, Benito M, Villagrasa M, Brianti E, Otranto D: ***Thelazia callipaeda*****: infection in dogs: a new parasite for Spain.** *Parasit Vectors* 2011, **4:**148.
13. Otranto D, Dutto M: **Human Thelaziasis, Europe.** *Emerg Infect Dis* 2008, **14:**647–649.
14. Otranto D, Lia RP, Cantacessi C, Testini G, Troccoli A, Shen JL, Wang ZX: **Nematode biology and larval development of** ***Thelazia callipaeda*** **(Spirurida, Thelaziidae) in the drosophilid intermediate host in Europe and China.** *Parasitol* 2005, **131:**847–855.
15. Otranto D, Brianti E, Cantacessi C, Lia R, Màca J: **The zoophilic fruitfly** ***Phortica variegata*****: morphology, ecology and biological niche.** *Med Vet Entomol* 2006, **20:**358–364.
16. Otranto D, Eberhard ML: **Zoonotic helminths affecting the human eye.** *Parasit Vecors* 2011, **4:**41.
17. Otranto D, Lia RP, Buono V, Traversa D, Giangaspero A: **Biology of T*helazia callipaeda*** **(Spirurida, Thelaziidae) eyeworms in naturally infected definitive hosts.** *Parasitol* 2004, **129:**627–633.
18. Lia RP, Traversa D, Agostini A, Otranto D: **Field efficacy of moxidectin 1 per cent against** ***Thelazia callipaeda*** **in naturally infected dogs.** *Vet Rec* 2004, **154:**143–145.
19. Rossi L, Rigano C, Tomio E, Frassetto D, Ferroglio E: **Use of sustained-release moxidectin to prevent eyeworm (*****Thelazia callipaeda*****) infection in dogs.** *Vet Rec* 2007, **161:**820–821.
20. Bianciardi P, Otranto D: **Treatment of dog thelaziosis caused by Thelazia callipaeda (Spirurida, Thelaziidae) using a topical formulation of imidacloprid 10 % and moxidectin 2.5 %.** *Vet Parasitol* 2005, **129:**89–93.
21. Ferroglio E, Rossi L, Tomio E, Schenker R, Bianciardi P: **Therapeutic and prophylactic efficacy of milbemycin oxime (Interceptor) against** ***Thelazia callipaeda*** **in naturally exposed dogs.** *Vet Parasitol* 2008, **154:**351–353.
22. Bussiéras J, Chermette R, Seillier A-M: **Quelques parasitoses canines exceptionelles en France. II - Un cas de conjonctivite parasitaire du chien, due à** ***Thelazia*** **sp.** *Prat Méd Chir Animal Compagn* 1996, **31:**83–85.
23. Kozlov DP: **The life cycle of nematode** ***Thelazia callipaeda*** **parasitic in the eye of the man and carnivores.** *Doklady Akademy Nauk SSSR* 1962, **142:**732–733.

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